

CLAIMS

1. An integration and expression plastid vector competent for stably transforming the plastid genome where growth is inhibited by an antibiotic-free phytotoxic agent which comprises an expression cassette which comprises as operably joined components, a 5' part of the plastid DNA sequence inclusive of a spacer sequence, a promoter operative in said plastid, a DNA sequence encoding a detoxifying enzyme or protein acting as a selectable marker which is capable of detoxifying said antibiotic-free phytotoxic agent in the cells to the corresponding nontoxic compound, at least one restriction site for the insertion of a heterologous target gene, a transcription termination region functional in said plastid, and the 3' part of a plastid DNA sequence inclusive of the spacer sequence.

2. The vector of claim 1 wherein a heterologous DNA sequence coding for a molecule of interest is inserted in one of the restriction sites.

3. The vector of claim 1 wherein said vector further comprises a ribosome binding site and a 5' untranslated region (5' UTR).

4. A vector of claim 1, wherein the antibiotic-free phytotoxic agent is a phytotoxic aldehyde and the detoxifying enzyme or protein is a aldehyde dehydrogenase capable of detoxifying said phytotoxic aldehyde.

5. A chloroplast vector of claim 2 wherein the molecule of interest is a polypeptide.

6. A vector of claim 3 or claim 4, wherein plastid is tobacco chloroplast.

7. A chloroplast vector which is described in Figure 1

8. A vector of claim 4 competent for stably transforming the chloroplast genome where growth is inhibited by a phytotoxic aldehyde which is selected from the group consisting of acetaldehyde, formaldehyde, propionaldehyde, butyraldehyde and betaine aldehyde.

9. An integration and expression plastid vector competent for stably transforming the plastid genome where growth is inhibited by a phytotoxic aldehyde which comprises an expression cassette which comprises as operably joined components, a 5' part of the plastid DNA sequence inclusive of a spacer sequence, a promoter operative in said plastid, a DNA sequence encoding betaine aldehyde dehydrogenase (BADH) as a selectable marker which is capable of detoxifying said phytotoxic aldehyde in the cells to glycine betaine, a heterologous DNA sequence which codes for

1 a molecule of interest, a transcription termination region functional in said plastid, and a 3' part of a plastid DNA sequence inclusive of the spacer sequence.

10. A stably transformed plant which comprises a chloroplast which has been stably transformed with a vector of claim 8 or claim 9, or the progeny thereof.

11. The stably transformed plant of claim 10, wherein the plant is a solanaceous plant
6 edible for a mammal.

12. The stably transformed plant of claim 10, wherein the plant is a crop plant edible for a mammal.

13. A stably transformed plant of either claim 11 or claim 12, wherein the mammal is a human.

11 14. A stably transformed plant of claim 10, wherein the plant is a monocotyledonous plant, selected from the group of rice, wheat, grass, rye, barley, oat, or maize.

rule 126 14. A stably transformed plant of claim 10, wherein the plant is a dicotyledonous plant, selected from the group of soybean, peanut, grape, sweet potato, pea, canola, tobacco, tomato or cotton.

16 15. A stable transformed plant of claim 10, wherein the plant is a tobacco, tomato, potato, rice, brassica, cotton, maize or soybean.

16. A stable transformed plant of claim 10, wherein the plant is a homoplasmic plant.

17. A vector of any one of claims 2-9, wherein the selectable marker is driven by a promoter in green and non-green tissues selected from the group consisting of the 16SrRNA
21 promoter, the psbA promoter, the alpB promoter, or the accD promoter.

18. A method for transforming the plastid genome of a plant cell, which method does not require selection for successful transformants by the detection of antibiotic resistance, said method comprising introducing into cells of a plant species whose growth is inhibited by an antibiotic-free phytotoxic agent, an expression cassette which comprises as operably linked components, a 5' part
26 of a plastid DNA sequence inclusive of a spacer sequence, a promoter operative in said plastid, a DNA sequence encoding a detoxifying enzyme or protein acting as a selectable marker for transgenic plant cells and capable of detoxifying said phytotoxic agent in the cells to the corresponding nonoxic compound, a heterologous target DNA sequence, a transcription termination region functional in said plant chloroplast cells, and the 3' part of the plastid DNA sequence inclusive of a spacer sequence.

- 1 ²⁰~~19~~. The method of claim 18 wherein the heterologous target DNA sequence codes for a molecule of interest.
- ²¹~~20~~. The method of claim 18 wherein the antibiotic-free phytotoxic agent codes for a phytotoxic aldehyde and the detoxifying enzyme or protein is a aldehyde dehydrogenase capable of detoxifying said phytotoxic aldehyde.
- 6 ²~~21~~. The method of claim 18 wherein the phytotoxic aldehyde which is selected from the group consisting of acetaldehyde, formaldehyde, propionaldehyde, butyraldehyde and betaine aldehyde.
- ³~~22~~. A method of claim 18, wherein said method further comprises culturing said plant in a plant growth medium comprising said phytotoxic aldehyde, and selecting transformed plant cells
- 11 ⁴~~23~~. A method of claim 22, wherein said method further comprises regenerating a transformed plant from said transformed plant cells.
- ⁵~~24~~. A method of claim 20 wherein said phytotoxic aldehyde is betaine aldehyde and the aldehyde dehydrogenase is betaine aldehyde dehydrogenase (BADH).
- 16 ⁶~~25~~. A method of claim 24, wherein said DNA sequence is from plants such as sugar beet, or spinach.
- ⁷~~26~~. A method of claim 24, wherein said DNA sequence is from a microorganism such as E. Coli.
- ⁸~~27~~. A method of claim 18, wherein the promoter is selected from a group consisting of
- 21 ⁹~~28~~. A vector of claim 2, 3 or 17, wherein the phytotoxic agent is selected from a group consisting of an herbicide listed in Table 18.4 of Molecular Biotechnology by Glick, light, betaine aldehyde and polyethylene glycol, and wherein the detoxifying agent is selected from a group consisting of an enzyme or protein capable of detoxifying said herbicide, the chlB gene, betaine
- 26 ¹⁰~~29~~. A method of any one of claims 18-27, where the expression cassette further comprises a ribosome binding site (rbs) and a 5' untranslated region (5'UTR) to enhance expression.